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### JOHNE'S DISEASE (PARATUBERCULOSIS) OF LIVESTOCK

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#### DESCRIPTION AND DISTRIBUTION

Johne's disease is an incurable, infectious, bacterial dysentery affecting principally cattle, but it may also affect horses, sheep, deer, and goats. It is also called paratuberculosis, bacterial enteritis, chronic hypertrophic enteritis, and chronic pseudotuberculosis enteritis. It was given the name of "pseudotuberculosis enteritis" by

some of the early investigators.

Johne's disease was first studied bacteriologically in Dresden, Germany, in 1895 by Johne and Frothingham. It has since been found in India and many countries of Europe, including the Channel Islands. It has also been diagnosed in many sections of the United States, and there is no doubt that the infection has spread to virtually every State. It is not definitely known when the disease first made its appearance in the New World, but it must have been more than a quarter of a century ago; and, like tuberculosis, it was brought in with shipments of cattle from Europe.

#### CAUSE OF THE DISEASE

The disease is caused by the *Mycobacterium paratuberculosis*, an aerobic, sporeless, nonmotile, and alcohol-fast bacillus, which is usually found in nests and in large clumps in the affected parts of the intestinal mucous membranes, and in the mesenteric lymph glands. The organism is 0.5 to 1.5 microns long and 0.3 to 0.5 micron in width, and, like the tubercle bacillus, it stains more or less irregularly. It grows with difficulty on any culture medium, but shows a tendency to produce the best growth on a glycerinated medium containing killed-tubercle-bacilli extract and 10 per cent normal cow serum.

In making cultures, tissues from suspected animals are cut in small pieces, preferably square. The pieces are submerged in 20 to 30 per cent fresh antiformin from 10 to 30 minutes. Planting of the pieces of tissue on to a suitable medium should not be done until at least 10 minutes of treatment has been completed and should be continued through 30 minutes or more. When these sections of tissue are planted after varying periods of treatment, it is possible that a growth may be established on a number of the planted tubes.

It is not absolutely necessary to wash the tissue free of antiformin, but if the tissues are washed, sterile, physiological, saline solution should be used in this procedure. It has been found that Johne's bacillus will withstand successfully four days' treatment with

50 per cent antiformin.

#### FORWARDING SPECIMENS FOR LABORATORY DIAGNOSIS

Specimens from suspected cattle should be covered with powdered borax, when forwarded for laboratory examination. Sections of any portion of the intestines showing inflamed areas are likely to con-

tain the bacilli of Johne's disease.

In the forwarding of specimens of intestines, the sections showing suspected lesions should be removed and only the excess of alimentary contents squeezed out and discarded. Both ends of this section should be tied with ordinary string. The specimen should then be covered with a thick layer of powdered borax and wrapped in clean gauze or muslin. It should then be placed in a container and sent to the laboratory for diagnosis accompanied by a letter giving full information regarding the species, age, history, symptoms, and lesions of the animal from which the specimen was taken.

The enlarged mesenteric glands, ileocecal valve, and rectum are other parts of the body in which the organisms are frequently harbored. In suspected animals, scrapings from the submucous and mucous membranes of the rectum very often show the presence of

Johne's disease.

#### MODE OF INFECTION

The disease is spread from herd to herd by diseased animals being introduced into clean herds, by diseased animals grazing on community pastures with healthy animals, and by drinking from slow-running, contaminated brooks. It is spread from animal to animal within a herd by bacilli expelled from the diseased animal's body with the feces which contaminate the feed and water. From a practical viewpoint infection of an animal occurs only when the causal agents are taken into the body by the mouth with food or water, unless the calf is infected in the uterus, a cause which has not been definitely determined.

In planning for the control and eradication of the disease it is necessary to give special attention to such possible sources of infection as ponds, sloughs, slow-running streams, water troughs, and feed boxes. The practice of feeding hay and fodder in field lots on the ground, where it may become contaminated by dried feces, should be discouraged. Just how long a pasture may remain infected has not yet been definitely determined. The time obviously may be ex-

pected to vary under different conditions.

#### PERIOD OF INCUBATION

Although the period of incubation is not known, it is believed that some animals may become infected with Johne's disease when only a few weeks old and the disease not manifest itself to the extent that any symptoms are noticeable until the animals are 3 or 4 years old. Certainly it is a very slow-developing disease, and for that reason is very difficult to combat.

The evidence at hand indicates that all blood lines and all breeds of cattle are susceptible to infection, but the disease appears to be

more fatal in some breeds than in others.

#### SYMPTOMS

The first symptoms usually observed in a case of Johne's disease are a temporary loss of appetite, and in cows a radical diminution of the milk yield. These symptoms are usually followed by intermittent diarrhea or by progressive diarrhea which fails to respond to the usual medicinal agents for the correction of such conditions.

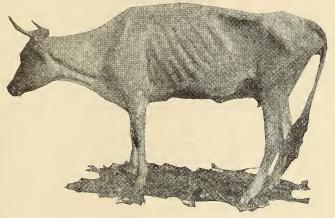


FIGURE 1.—Attitude of a cow in the last stages of Johne's disease

The coat becomes rough and the animal looks unthrifty. The appetite may be regained after the first few days and thereafter remain normal. The animal may drink large quantities of water. While some writers on the subject of Johne's disease have not observed any elevation of temperature, others claim that the temperature is sometimes elevated several degrees. In the last stages of the disease (fig. 1) the animal becomes greatly emaciated and dies as the result of exhaustion due to superpurgation. Death is often hastened by parturition and has been observed in valuable purebred herds when cows are placed on official test for production. This may be readily explained since in producing large quantities of milk the vitality of the animal is lessened, thereby making it an easy victim to the disease.

#### TREATMENT

No satisfactory treatment for Johne's disease is known. In the earlier stages when the diarrhea is intermittent, temporary relief

is sometimes obtained by restricting the feed and placing the animal on a dry diet. Care should be exercised to isolate the suspected cases until such time as a positive diagnosis can be made. Millions of the bacteria which cause the disease are expelled in the feces of the infected animals; therefore the droppings from suspected and diseased animals should be buried in lime or disposed of in a cultivated field not accessible to susceptible animals. Treatment should be restricted to preventive measures, as cures can not be expected.

#### THE JOHNIN TEST

The johnin test is applied to individual animals or herds of cattle in order to ascertain the presence or absence of Johne's disease. It is used in the same manner as the tuberculin test is used in the detection of tuberculosis, except that the injections of johnin are made intravenously, into the jugular vein.

The animals should be arranged as usual in the barn and, through-

out the test, water should be withheld from them.

Before the application of the test three preinjection temperatures should be taken at 2-hour intervals to be certain that the animals' temperatures are normal. All animals registering a temperature of 103° F. or more should be withdrawn from the test. In the writers' experience it has been noted that in very warm weather the test should not be applied, as conflicting results are often obtained from abnormal temperatures. When tests are made under such circumstances they should be given at sundown and continued throughout the night. Under good weather conditions the test is made in accordance with the method on the following record form.

The form below shows the record of animals tested with johnin and a comparison of the range in temperature between a healthy

cow (No. 62) and two diseased cows (Nos. 44 and 35).

Post-mortem lesions noted in cows 44 and 35 were typical of Johne's disease and microscopic examinations were positive for acid-fast organisms.

Temperature records and results of testing three cows with johnin, 5 cubic centimeters injected into each cow at 6 a.m.

(Temperatures are degrees Fahrenheit above 100°)

Cow No.		einject perat at—			Postinjection temperatures at—								Remarks				
	2 a. m.	4 a .m.	6 a. m.	6.30 a. m.	7 a. m.	8 a. m.	9 a. m.	10 a. m.	11 a. m.	12 m.	1 p. m.	2 p. m.	3 p. m.	4 p. m.	5 p. m.	6 p. m.	
44	2. 4	2. 0	0.8	1.6	1.4	1.4	2. 2	2. 6	4. 1	4. 0	4.4	3.8	3. 6	2.8	2.8	2.4	Reactor killed.1
35	2. 2	2. 2	2. 6	2. 4	2.6	2.8	2.8	3.8	4. 3	5. 3	4.5	4. 4	4. 2	4.4	4. 5	4. 4	Reactor died.1
62	2. 0	1.4	1.8	1.8	1.4	1.2	1.5	1. 2	. 7	1, 6	1. 9	2. 0	2. 0	1. 7	1.6	1. 5	Nega- tive.

<sup>1</sup> Acid-fast organisms found microscopically.

Immediately after the third preinjection temperature has been taken johnin is applied to all the animals intravenously. Postinjec-

tion temperatures should be taken, beginning one-half hour later and continued every hour for 12 hours. Reactions may take place in diseased animals three to eight hours after the test has been applied. If there is 1.5° F. or more rise in temperature above the highest preinjection temperature the animal should be classed as a reactor. An animal having a rise in temperature, but not sufficiently high to class as a reactor, should be regarded as suspicious and retested at a later date.

#### USE OF AVIAN TUBERCULIN AND JOHNIN

In the early study of Johne's disease and up to comparatively recent times avian tuberculin has been used in herd tests owing to the difficulty of culturing the Johne's bacillus for the preparation of

johnin.

Tuberculin prepared only from avian tubercle bacilli is used for such testing purposes. However, tuberculous animals may react to this test also, in which case the Johne's disease should be differentiated by an application of mammalian tuberculin. Subcutaneous injections of avian tuberculin in the presence of Johne's disease produces an elevation of temperature, diminished milk yield, chills, depression, and diarrhea. However, the use of johnin in the diagnosis of Johne's disease gives a more distinct reaction than the avian tuberculin.

The term "johnin" has been coined to apply to the biological product prepared by growing the Johne's bacillus on liquid medium and then sterilizing and filtering the finished product in precisely the same manner as tuberculin is prepared. In fact the Johne's bacillus is used in the preparation of johnin in the same manner that the tubercule bacillus is used in the production of tuberculin. It has the same physical appearance as tuberculin, but requires a much longer time to prepare because the growth on the liquid medium does not develop rapidly—in fact the cultures must be incubated con-

tinuously for from five to nine months.

Johnin does not cause any thermal reaction in tuberculous cattle. In the United States a large number of herds have been tested with johnin injected into the jugular vein. As already explained, johnin gives a thermal reaction in the presence of Johne's disease, the temperature rising 1.5° F. or more above the normal temperature of the animal. By means of this test, animals harboring Johne's disease react a few hours after inoculation. Reactors may show or may not show clinical symptoms. Intradermic inoculations of the concentrated johnin into the caudal fold of cattle have frequently produced large swellings. These animals had previously given positive reactions to the intravenous tests applied to them.

#### POST-MORTEM APPEARANCE

In advanced cases the carcass will be emaciated and serous fluid may be present in the abdominal cavity in old or extensive cases. A specific enteritis in both the large and small intestines may be present, though not always. The middle section (ileum) or small intestine is the part of the bowels most often affected. In mild cases it may be difficult to detect any abnormal condition in the intestines, but in old, chronic cases the walls of some portion of the small intestines will appear thickened. When this is opened the mucous lining presents an abnormal, wrinkled, or corrugated appearance.

(Fig. 2.)

This condition should not be confused with the normal furrows found in healthy intestines of cattle. There is little or no general inflammation in any section of the bowels, and in this respect differs from other conditions which produce diarrhea. The mesenteric lymphatic glands are enlarged, and on their being incised a milk-like fluid escapes.

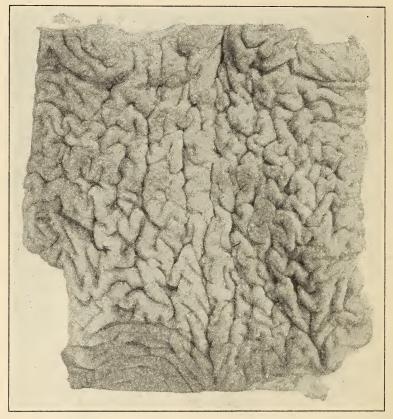


Figure 2.—Johne's disease in intestine of cow. Section of intestine showing thickened mucous membrane and corrugations

#### MICROSCOPIC FINDINGS

Specimens taken from animals reacting to the johnin test have been examined in numerous cases, and in a majority of them the acid-fast organism causing Johne's disease was demonstrated microscopically.

Usually, the organisms are to be found in clumps or nests in the inner walls of the intestines, and it has been often necessary to examine smears taken from as many as 25 to 50 areas on or under the

mucosa of the intestines before the organisms could be found and a positive diagnosis of the disease made. Negative findings do not

preclude the existence of the disease.

The organisms are found unequally distributed in various sections of the intestines, and even though the tissues are inflamed and thickened, these causal agents are not always exhibited therein. Certain parts of the intestines are found to be the preferred nesting places for the organisms, and the locations in which they have been demonstrated more often than in other sections are as follows: Jejunum, ileum, ileocecal valve, rectum, and the mesenteric lymph glands.

In the examination of feces from suspected animals, the best results are obtained by the use of antiformin, but a definite diagnosis can not always be made. The value of antiformin lies in the fact that it acts as a solvent of tissue cells and of all bacteria that are not acid-fast, but it has no such action on such acid-fast bacteria as the

tubercle and paratubercle bacilli.

Mixing 20 parts of the scrapings from the intestine to be examined with 20 parts of antiformin and 60 parts of distilled water, shaking thoroughly for 20 minutes, and then centrifuging will cause any acid-fast bacteria that may be present to accumulate at the bottom. This sediment is then stained with Ziehl-Neelsen's method in the usual manner to determine the presence or absence of acid-fast organisms. It is obvious that microscopic examination is the surest means of diagnosis.

#### DIFFERENTIAL DIAGNOSIS

As has already been stated, the principal infection with which Johne's disease may be confused is tuberculosis. However, the latter disease may be readily distinguished from the former through the application of the customary tuberculin test, which will produce a reaction in the tuberculous animal but will have no effect on an

animal suffering with Johne's disease.

Various invasions of gastrointestinal parasites may also be mistaken for Johne's disease, but in those instances a microscopic examination of the feces will usually result in the discovery of ova, larvæ, or adult parasites from which a diagnosis may be established. However, the presence of such parasites does not necessarily exclude the presence of Johne's disease, as parasitism and Johne's disease may exist simultaneously. On the other hand, it is extremely rare to find both Johne's disease and tuberculosis existing in the same animal.

#### HISTORY OF AN AFFECTED HERD

Some idea of the losses which Johne's disease may cause can be gained from the history of a fine herd of more than 200 dairy cattle that has been brought to the attention of the Bureau of Animal

Industry.

A study of the herd record indicated that infection had been present for a number of years, and revealed the startling fact that approximately 10 per cent of this large herd died annually of Johne's disease. These deaths occurred in cows of from  $2\frac{1}{2}$  to 9 years of age.

A consultation was held by bureau and State veterinarians concerning what procedure should be followed to control and eradicate the disease in this valuable herd. It was agreed that the johnin test should be applied, which was done. For convenience the herd was divided into two lots of approximately 100 animals each, and the prescribed dose of johnin was injected into the jugular vein of each animal. The reading of temperatures was begun 30 minutes after the injection of the diagnostic agent and was continued every hour until 12 temperatures had been recorded. As a result of this test 45.4 per cent of the herd were classed as suspects or reactors. There were included in this lot of animals 21 calves ranging in age from 3 weeks to 6 months, only 2 of which successfully passed the test. Six of the reacting animals, including two young calves, were at once slaughtered and typical lesions were found in each case. Microscopic examination revealed the presence of acid-fast organisms. Further steps were taken to control and eradicate the disease in this herd and notable progress has been made.

#### INDEMNITY FOR JOHNIN REACTORS

The Bureau of Animal Industry has been granted authority by Congress to pay indemnity to cattle owners for animals that are slaughtered because of reaction to the johnin test. These payments are made on the same basis that indemnity is paid on tuberculous cattle.

Indemnity funds have been available for this purpose since July 1, 1927, from which a number of livestock owners have received benefits. Further details concerning the payment of indemnity on cattle reacting to Johne's disease can be obtained by writing the livestock officials of your State or the United States Bureau of Animal Industry, Washington, D. C.